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Unusual Presentation of Disseminated *Mycobacterium kansasii* Infection in Renal Transplant Recipients and Rapid Diagnosis Using Plasma Microbial Cell-free DNA Next-generation Sequencing

Tosin Ogunsiakan, MD,[1] Kristen D. Fajgenbaum, MD,[2] Thomas B. Montgomery, MD,[1] Gautam M. Phadke, MD,[3] and Kiran Gajurel, MD[4]

INTRODUCTION

Mycobacterium kansasii (MK) is an environmental nontubercular mycobacterium (NTM) that can occasionally cause human infections. The incidence of MK infection varies by geographic location, but in general, it is much less prevalent than *Mycobacterium avium* complex (MAC).[1,2] An earlier survey showed an incidence of MK infection of 0.52/100 000 population (compared with 3.2/100 000 population for MAC) in the United States.[1] MK infection is uncommon among solid organ transplant (SOT) recipients even though the incidence of NTM infection in this group is higher than in the general population.[2-4] Several studies have demonstrated a very low incidence of MK infection (anywhere from none to <1% incidence) in SOT.[5-18] In a 2016 review of NTM in SOT, Abad and Razonable[19] found 304 patients with NTM infection in SOT recipients.[19] Most of NTMs occurred in kidney transplants reflecting a higher relative volume of kidney transplants performed. MAC and *Mycobacterium abscessus* accounted for 127 (42%) cases. MK accounted for only 35 (12%) cases. More than two thirds of MK cases (24 cases) were reported in kidney transplant recipients. This was followed, in descending order, by heart transplant (9 cases) and lung transplant recipients (2 cases). Among kidney transplant recipients, MK was found to be the second most common cause of NTM (16% of NTM) behind *Mycobacterium chelonae* but was more common than MAC in 2 recent reviews.[19,20] MK was the most common NTM in heart transplants, where it contributed to 9 (27%) of 33 NTMs.[19] MK has rarely been reported in liver recipients.[21]

The low incidence of MK in SOT and time-consuming traditional mycobacterial cultures make it particularly challenging to diagnose this infection in a timely manner. In this case series, we report 2 unusual presentations of disseminated MK infection in kidney transplant recipients that were diagnosed rapidly by Karius, a plasma-based microbial cell-free DNA(mcfDNA) next-generation sequencing (NGS) test.

PATIENTS AND METHODS

After collection of whole blood from patients, it is centrifuged, and mcfDNA is extracted and NGS performed at Karius Inc (Redwood City, CA). Human sequences are removed, and the remaining nonhuman reads (mcfDNA) are aligned to a curated database of >1400 organisms. McfDNA detected above a predefined statistical threshold are reported and quantified in molecules per microliter (MPM).

Because the study involved case reports, our institutional review board determined that the study met criteria for exempt status and granted waiver of authorization.

Case 1

A 31-y-old female individual on a stable regimen of tacrolimus, mycophenolate, and prednisone (10 mg/d) presented 1 y after kidney transplant with a week-long history of fever up to 101 °F along with pain and swelling in the anterior neck in the midline, difficulty swallowing solid food, raspy voice, and a 5 Ib weight loss. She also had worsening pain and swelling causing restricted movement of her right thumb and

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[1] *Department of Medicine, Carolinas Medical Center, Atrium Health, Charlotte, NC.*
[2] *Department of Dermatology, University of North Carolina, Chapel Hill, NC.*
[3] *Metrolina Nephrology, Charlotte, NC.*
[4] *Division of Infectious Diseases, Carolinas Medical Center, Atrium Health, Charlotte, NC.*
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Correspondence: Kiran Gajurel, MD, Division of Infectious Disease, Carolinas Medical Center, Atrium Health, 1225 Harding Place, Ste 2100, Charlotte, NC 28204. (kgajurel@yahoo.com; kiran.gajurel@atriumhealth.org).

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left third finger for about 2 wk along with several months of intermittent bilateral knee pain and swelling that she attributed to her lupus. Computed tomography (CT) of the neck showed a 4.5 × 4.6 cm multilocular cystic swelling with rim enhancement in the infrahyoid soft tissue in the anterior neck consistent with an infected thyroglossal cyst. She was started on empiric vancomycin, cefepime, and dexamethasone. Aspiration of the neck swelling performed on postadmission day (PAD) 2 revealed purulent fluid, and fluorescent auramine-rhodamine stain showed 4+ acid-fast bacilli (AFB). The HIV antigen/antibody test was negative. CT chest was negative for lung lesions. The patient did not have risk factors for tuberculosis, but because of the involvement of the neck and fingers, disseminated mycobacterial infection was suspected. The patient was started on rifabutin (instead of rifampin to minimize drug interaction with tacrolimus), isoniazid with B6, pyrazinamide, ethambutol, and azithromycin on PAD 4 to target *Mycobacterium tuberculosis* (MTB) and MAC. Vancomycin, cefepime, and dexamethasone were discontinued. Mycophenolate dose was reduced because of disseminated infection, and tacrolimus dosing was adjusted because of drug interaction with rifabutin.

The fluid in the neck swelling reaccumulated rapidly and required reaspiration on PAD 8. The fluorescent auramine-rhodamine stain was again positive (2+ AFB). Despite repeated aspiration, the neck swelling got worse (Figure 1) and ruptured in the central area. Mycobacterial culture from the first aspirate grew AFB on PAD 8. MAC and MTB DNA probes on the culture were negative, and the culture did not turn characteristic orange until 21 d later (PAD 29) when MK DNA probe was done to confirm it as MK. The mycobacterial culture from the second aspirate grew AFB after 16 d on PAD 24 and was identified as MK 14 d later (on PAD 38) based on morphology (characteristic color). In contrast, the Karius test was sent on PAD 5 and resulted positive for MK at 284 MPM (reference <10) in 4 d on PAD 9 (Table 1). The Karius test also detected *Torque teno* virus 15 and *Peptoniphilus harei* of unclear significance. Although the Karius test enabled earlier diagnosis of MK, pyrazinamide and azithromycin were not stopped until the MTB and MAC probes were negative on PAD 11. Rifabutin, isoniazid with B6, and ethambutol were continued.

On PAD 9, the patient also required debridement of the right thumb. Purulent drainage was obtained, but cultures were not sent. Ultrasound of the left third finger showed fluid in the flexor tendon sheath, and clinically, it was consistent with tenosynovitis. It remained stable and did not require debridement.

FIGURE 1. Rapidly enlarging thyroglossal cyst despite repeated aspiration.

The patient started doing better until about 8 wk into treatment when she was admitted to another center with a week-long history of right-sided abdominal pain and was found to have a right psoas abscess measuring 8.4 × 4.7 × 4.7 cm. An intra-abdominal drain was placed, and the aspirated fluid was 4+ AFB positive on auramine-rhodamine stain and grew AFB on solid medium that was initially identified as MAC by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). MAC probe was, however, negative, and 16S rRNA and rpoB gene sequencing at the reference laboratory confirmed MK. Later, MK DNA probe was also positive. The subculture from liquid medium had 2 colony morphologies consistent with MAC and MK. The liquid medium was thought to be cross-contaminated with MAC. The antimycobacterial regimen was not changed, and the patient continued to get better. Figure 2 summarizes the timeline of events and diagnostic tests.

Case 2

A 59-y-old male individual presented 3 y and 8 mo after kidney transplant with a 4-mo history of malaise and unintentional 40 Ib weight loss, along with pain, stiffness, and mild swelling of bilateral interphalangeal and metacarpal joints, both wrists, elbows, and knee joints. The left knee was particularly painful, and although there was no frank effusion, it was warm and exquisitely tender with restricted movement. He complained of dyspnea and denied fever; however, on admission, he was febrile to 101 °F. Chest imaging did not show pneumonia, but transthoracic echocardiogram was notable for moderate to large pericardial effusion. He had been on a stable regimen of belatacept, mycophenolate, and prednisone (5 mg/d). He was started on empiric cefepime and vancomycin, but the antibiotics were stopped on PAD 3 as there was no overt infection. He was also noted to have a dry scab (<1 cm in diameter) in the left upper extremity overlying an old nonfunctional Gore-Tex arteriovenous graft (AVG) that had been placed 4 y prior with intermittent, scant, clear drainage for 4 mo. Ultrasound of this area showed a subcentimeter superficial fluid collection. There was no cellulitis or tenderness. The patient continued to have fevers and arthralgia/synovitis, especially in the knees. X-ray of the right elbow and bilateral hands and magnetic resonance imaging of the lower extremities showed mild degenerative changes. Microbiological workup including blood cultures, the HIV antigen/antibody test, hepatitis B surface antigen, hepatitis C antibody, serum cryptococcal antigen, urine histoplasma antigen, serum blastomyces antigen, serum coccidioides antibody, respiratory pathogen panel including severe acute respiratory syndrome coronavirus 2 polymerase chain reaction (PCR), malaria smear, serum 1.3 beta-D-glucan, serum parvovirus PCR, plasma Epstein-Barr virus PCR, and pericardial fluid AFB, fungal, and bacterial cultures were all negative. Rheumatoid factor was weakly positive at 15.9 IU/mL (reference <14.1 IU/mL), but antinuclear antibody and cyclic citrullinated peptide immunoglobulin G were negative. Colchicine was added, and prednisone was increased to 40 mg/d, but there was no clinical improvement. Because of the persistent fever, he was restarted on vancomycin and aztreonam (followed by ceftriaxone and then cefepime) along with doxycycline on PAD 9.

TABLE 1.

Rapid diagnosis of *M kansasii* infection using plasma microbial cell-free DNA NGS

Specimen	Specimen collection, PAD	No. of days required for AFB growth (PAD)	No. of days required for *M kansasii* identification (PAD)	Method of *M kansasii* identification	Comments
Case 1					
Thyroglossal cyst aspirate AFB culture	2	6 d (8)	27 d (29)	*M kansasii* DNA probe	Not viable for susceptibility
Thyroglossal cyst aspirate AFB culture	8	16 d (24)	30 d (38)	Morphology	Not viable for susceptibility
Psoas abscess AFB culture[a]	60	Unknown	Unknown	16S rRNA and rpoB gene sequencing, *M kansasii* DNA probe and MALDI-TOF MS	Rifampin and clarithromycin sensitive, MAC was also isolated (thought to be a contaminant)
Plasma (Karius)	5	NA	4 d (9)	NGS	*Torque teno* virus 15 and *Peptoniphilus harei* were also identified
Case 2					
AVG AFB culture	14	13 d (27)	22 d (36)	*M kansasii* DNA probe	
Soft tissue around stent AFB culture	14	18 d (32)	28 d (42)	Morphology	
Blood AFB culture	16	19 d (35)	34 d (50)	Morphology	Rifampin and clarithromycin sensitive
Plasma (Karius)	12	NA	3 d (15)	NGS	Cytomegalovirus was also detected

[a]Tests done at another center (thus all the data are not available).

AFB, acid fast bacilli; AVG, arteriovenous graft; MAC, *Mycobacterium avium* complex; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; *M kansasii*, *Mycobacterium kansasii*; NA, not applicable; NGS, next-generation sequencing; PAD, postadmission day; rRNA, ribosomal RNA.

Positron emission tomography (PET) CT performed on PAD 13 showed hypermetabolic activity in the soft tissue along the AVG in the left upper extremity and the neck. The following day he underwent resection of the purulent AVG and the innominate vein stent. The fluorescent auramine-rhodamine stain of the AVG was 1+ AFB positive but that of the soft tissue stent was negative. The AVG AFB culture grew AFB on PAD 27, and MK was identified in 22 d (on PAD 36) by MK DNA probe when the culture turned characteristic orange. The AFB culture of the soft tissue was even more slow to grow and was identified as MK based on morphology on PAD 42 (Table 1).

Meanwhile, the Karius test was sent on PAD 12, a day before PET CT was done. It resulted positive for MK at 1314 MPM (normal <10) and for cytomegalovirus (CMV) at 225 MPM (normal <10) in 3 d (PAD 15). MK was not expected, and the Karius test was thought to be false-positive. The next day when the auramine-rhodamine stain of AVG came back positive for AFB, he was started on rifampin (later replaced by rifabutin to minimize drug interaction), isoniazid with B6, and ethambutol. Other antimicrobials were stopped after the PET CT, and the Karius test results were available. The AFB blood culture was also obtained before initiation of antimycobacterial therapy, and it also later grew MK, suggesting disseminated infection (Table 1). Figure 3 summarizes the timeline of events and diagnostic tests.

CMV in the Karius test was not considered significant, but during the course of treatment, he developed plasma CMV

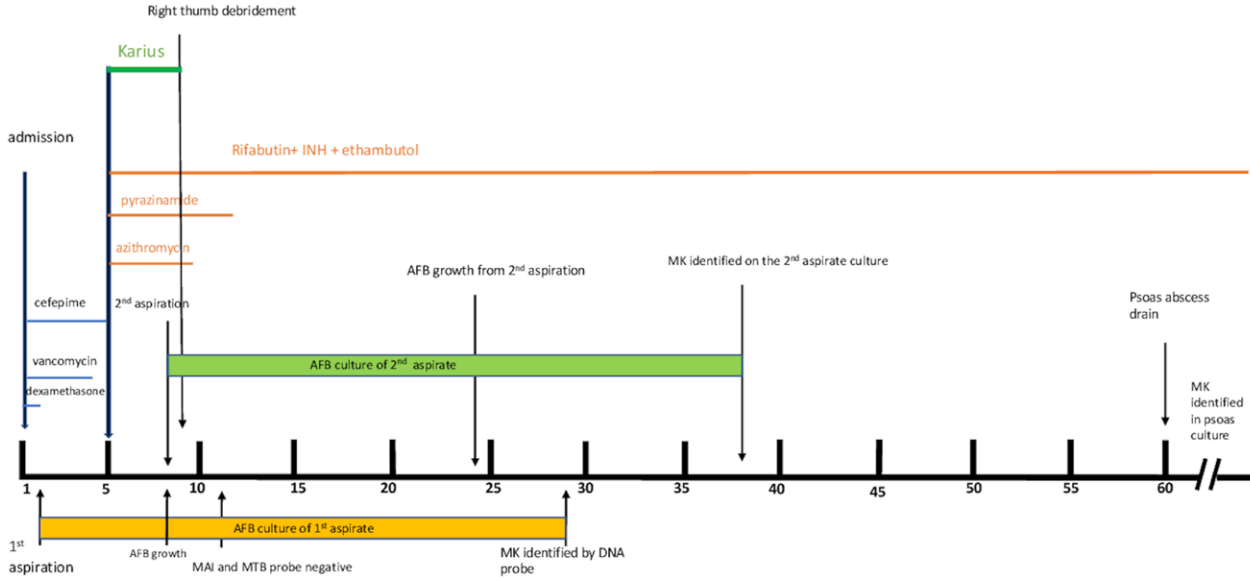

FIGURE 2. Timeline of events and diagnostics in case 1. AFB, acid fast bacilli; INH, isoniazid; MAI, *Mycobacterium avium* intracellulare infection; MK, *Mycobacterium kansasii*; MTB, *Mycobacterium tuberculosis*.

viral load of 5210 IU/mL and required valganciclovir treatment. Because of leukopenia and the need for valganciclovir therapy, betalacept and mycophenolate were switched to cyclosporine, and prednisone was tapered to 5 mg daily. The patient responded well to treatment with weight gain and improvement of synovitis, malaise, leukopenia, and pericardial fluid.

DISCUSSION

In the general population, MK is primarily a pulmonary infection, and extrapulmonary manifestation is rare.[22] In SOT, on the other hand, disseminated disease including extrapulmonary infection is not uncommon.[23] Both of our patients had unusual presentations of extrapulmonary disseminated MK infection. Even though musculoskeletal involvement is a fairly common presentation of MK infection in SOT, to our knowledge, MK infection of the thyroglossal cyst in SOT has not been reported before.[23] An MK-related psoas abscess, unlike a tubercular psoas abscess, is also an unusual finding and has been described sporadically in the non-SOT population.[24-29] Similarly, pericarditis and AVG infection (second patient) are uncommon manifestations of MK infection in SOT. (Although the pericardial fluid AFB culture was negative, the patient likely had pericardial infection in the setting of mycobacteremia). MK-related pericarditis in a kidney transplant recipient has been described once before, and there are 2 other reported cases of MK-related AVG infection in kidney recipients.[7,23,30] MK bacteremia, although described in advanced HIV infection, is also a rare finding in SOT.[31,32]

The diagnosis of MK infection in SOT can be delayed because of lack of clinical suspicion contributing to a failure to order AFB cultures on clinical specimens. This, in turn, is because of a low incidence of MK and nonspecific clinical presentation. Moreover, AFB cultures, especially of extrapulmonary sites, have suboptimal sensitivity. MK, in particular, is traditionally considered a "slow grower," and it usually takes >7 d (and up to 6 wk) to grow adequately for identification, thus leading to diagnostic delay.

In recent years, Karius plasma mcfDNA NGS has emerged as a promising rapid diagnostic tool in many infectious diseases.[33-37] Unlike traditional diagnostic tests, it is an unbiased technique that does not require prior knowledge of putative pathogens and circumvents the need for pathogen-specific tests. The Karius test enabled early diagnosis of MK infection in both the patients. In the first patient, Karius diagnosis was made 4 d after sample collection compared with 27 d on culture media, and in the second patient, Karius diagnosis was made in 3 d compared with 22 d on culture media. This helped in modifying empiric treatment in case 1 and in initiating MK-specific treatment in case 2 much earlier than the traditional cultures would have allowed. It is important to note that in both patients, AFB stains on the clinical specimens (thyroglossal cyst aspirate and AVG explant) were positive and thus increased the likelihood of mycobacterial infection. Even though epidemiologically tuberculosis was unlikely, there are several different pathogenic NTM species, each requiring a different therapeutic regimen. This makes it difficult to choose an empiric regimen based on a positive AFB stain. Growth in culture media is required for identification of NTM in most instances to ensure that the patient is on appropriate antimicrobial therapy while susceptibility is pending (which can take several weeks). This is particularly challenging for slow-growing NTMs like MK. It should also be noted that even after growth of AFB, it may take additional time for proper identification of mycobacterial species, as seen in our patients. In the first patient, AFB culture was positive on PAD 8 but was not identified as MK until PAD 29 when the culture turned characteristic orange and MK probe was performed. Similarly, in the second patient, AFB culture was detected on

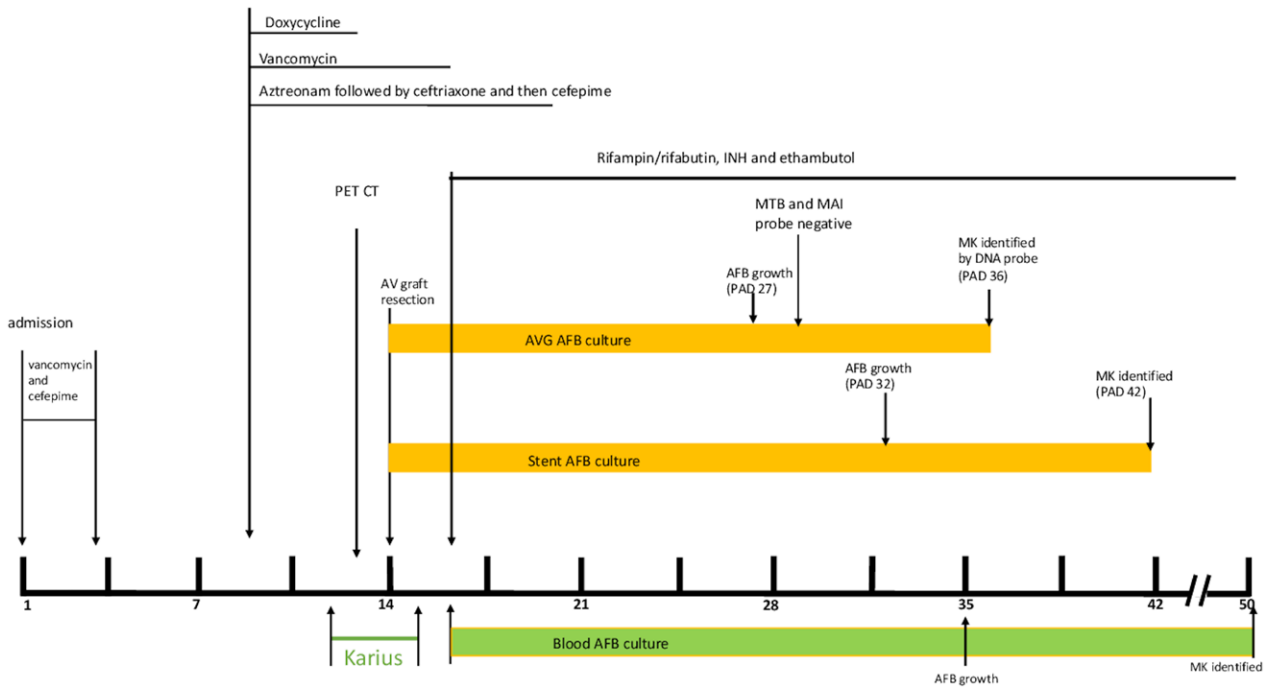

FIGURE 3. Timeline of events and diagnostics in case 2. AFB, acid fast bacilli; AVG, arteriovenous graft; CT, computed tomography; INH, isoniazid; MAI, *Mycobacterium avium* intracellulare infection; MK, *Mycobacterium kansasii*; MTB, *Mycobacterium tuberculosis*; PAD, postadmission day; PET, positron emission tomography.

PAD 27 but was not identified as MK until PAD 36. Other diagnostic tests like genomic sequencing and MALDI-TOF MS can expedite the diagnosis of NTM, but they still require growth of AFB in the culture media.[38-41] Genomic sequencing is available only in reference centers and cannot be performed readily. Although MALDI-TOF MS is used in many laboratories for the identification of mycobacteria, we were unable to use it because our laboratory is in the process of validating it for mycobacterial diagnosis.

It should be noted that the Karius test does not provide information on antimicrobial susceptibility and thus cannot replace traditional cultures. The Karius test also needs to be interpreted in context. The test can detect polymicrobial organisms that may not have clinical relevance, as seen in our first patient where *Torque teno* virus and *Peptoniphilus harei* were also detected.[42,43] Moreover, some of the potential pathogenic organisms can be difficult to ignore when detected even though they may not be clinically relevant. Incidental findings such as these may prompt unnecessary antimicrobial therapy.[43,44] In one study, the Karius test did not have a significant clinical impact likely because a number of patients already had a diagnosis through conventional testing[42]; however, judicious use of this test in a population with a high likelihood of infections that may be difficult to diagnose through conventional testing in a timely manner seems to be promising.[42,44] By enabling earlier diagnosis, it allows timely treatment and can potentially prevent progression of disease and decrease morbidity and mortality, but further studies are needed to confirm its positive impact on clinical outcome. Its utility is limited by the lack of availability of the test outside the United States. It is available in the United States, but individual institutions may have their own policies on its implementation, with some institutions requiring preapproval of the test by microbiology or infectious disease physicians.[42] The cost of the test ($2000 per test) can also be prohibitive, although it has been demonstrated to be cost effective in certain settings.[45] More studies are needed in SOT to evaluate its real-world cost-effectiveness.

In summary, these 2 cases highlight unusual presentations of disseminated MK in kidney transplant recipients and the usefulness of plasma mcfDNA NGS in early diagnosis and treatment. Plasma mcfDNA could be an important advantage because of the slow-growing nature of organisms like MK, where conventional testing takes weeks. This technology can be used as a complemental tool in the diagnostic workup of patients with difficult-to-diagnose infections.

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